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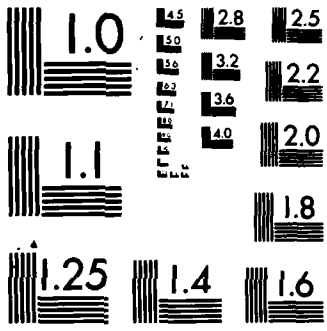

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SMOKING AND THE DIFFERENTIAL WHITE BLOOD CELL COUNT AS DETERMINED ON A TECHNICON H6000™ AUTOMATED BLOOD CELL ANALYZER

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SMOKING AND THE DIFFERENTIAL WHITE BLOOD CELL COUNT AS DETERMINED ON A TECHNICON H6000™ AUTOMATED BLOOD CELL ANALYZER

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SUMMARY

Objective

To determine the effect smoking has on the differential white blood cell count using a Technicon H6000 automated blood cell analyzer.

Approach

Blood samples from 2968 apparently healthy employees of the Naval Weapons Center (NWC), China Lake, California, were collected from January 1982 to November 1982 as part of an ongoing Occupational Health Program at NWC. All blood samples were collected between 8:30 and 11:00 a.m. to minimize hour-to-hour variation in the white blood cell counts. A Technicon H6000 automated blood cell analyzer interfaced to a VAX/750 computer was used to analyze the blood samples.

Results

A significant increase in number of all leukocyte cell types was observed in smokers (8177 cells per mm^3) as compared to nonsmokers (6319 cells per mm^3) ($p < 0.001$). The largest relative percent increase occurred in neutrophils (36%) and the lowest relative percent increase in eosinophils (14%). Smokers had a slight increase in mean percentage of neutrophils compared with nonsmokers, and a slight decrease in mean percentage of lymphocytes. Smoking also appears to have affected the platelet count. Both male and female smokers show a slight increase in their platelet count, 3.2% and 5.1% higher counts respectively, in comparison to nonsmokers.

Conclusion

With the use of the new Technicon H6000 blood cell analyzers which counts as many as 10,000 cells per sample, a more accurate picture of the effect smoking has on the leukocyte count is now available. Our study showed that cigarette smoking significantly increased all five cell types, including platelets. The mechanism by which this occurred is not known, but it is unlikely that a specific releasing or inducing factor is responsible for each of the different cell types. It is feasible that a non-specific factor is present in cigarette smoke (or its metabolites) which is responsible for this increase in the leukocyte and platelet counts observed in smokers.

Introduction

Cigarette smoking has been shown to be associated with an increase in total leukocyte counts.[1,2,3] However, the effect smoking has on the individual cell types is not clear. Corre et al. and others have examined the differential counts in smokers and nonsmokers and found increases in the percentage of neutrophils and decreases in the percentage of lymphocytes.[2,4] Noble et al. reported somewhat different results, finding a decrease in the percentage of neutrophils in smokers compared to nonsmokers.[5]

The inaccuracy in determining the differential-count percentages when examining a sample of only 100 or 200 cells could possibly account for this inconsistency.[6,7] We report the effect of smoking on the differential white blood cell count including platelets as determined on a Technicon H6000™ Automated Blood Cell Analyzer (Technicon Instruments Corp., Tarrytown, NY). This instrument examines 10,000 leukocytes in each sample, and determines cell types based upon size (as measured by light scatter) and intensity of biochemical reactions taking place within the cell. As a result of the larger number of cells being examined, differences between cell types can be detected with greater accuracy.[6]

Methods

Blood samples from 2968 apparently healthy employees of the Naval Weapons Center (NWC), China Lake, California, were collected from January 1982 to November 1982 as part of an ongoing Occupational Health Program at NWC. Approximately 70% (2033) of the participants were males and 30% (935) females. About 80% were between 25 and 54 years of age, and over 90% of the participants were white. Twenty-seven percent (803), were current smokers, and 73% (2145) were former or nonsmokers. All employees who volunteered were asked to give a 7 ml sample of blood and complete a questionnaire. The questionnaire obtained personal data including name, social security number, age, race, sex, a brief smoking history, and a limited NWC work history. The questionnaire was completed at the time the initial blood sample was drawn. All blood samples were collected between 8:30 and 11:00 a.m. to minimize hour-to-hour variation in the white blood cell counts.[8] Immediately after being drawn each sample was tagged with a bar code number which could be optically read by electronic equipment. This bar code number was also affixed to the questionnaire and a laboratory report form, then entered in a blood sample log. In addition, the Technicon H6000 was interfaced directly with VAX 11/750 computer. This procedure eliminated the need for manual data entry of blood analysis results, allowing rapid, accurate, and convenient linking of individual blood analyses with data concerning personal characteristics and job-related activities obtained from the questionnaire.

Before each daily operation, quality control measures were performed on the Technicon according to the manufacturer's recommendations.[9] Checks on instrument function and electronic components and background counts were performed daily prior to routine operation. In addition, a 25% systematic sample (N = 586) of total white blood cell counts performed using the Technicon were independently and blindly performed using a Coulter Counter® model ZBI (Coulter electronics, Inc., Hialeah FL). The Pearson product-moment-correlation between the two machines was r = 0.94.[10]

In reporting the 95% range of values for leukocyte cell types in smokers and nonsmokers, the 2.5 and 97.5 percentile values were used.[11, 12] This procedure does not involve any assumptions regarding the distributions other than they be continuous.

Study subject participation including all procedures were in accordance with the rules and regulations protecting human subjects prescribed by the Under Secretary of the Navy.

Results

The frequency distributions of leukocytes by cell type in smokers and nonsmokers are shown in Figures 1-5, and the corresponding means and percent differences according to smoking status are shown in Table 1. The sum of all leukocyte cell types was greater in smokers than in nonsmokers ($P < 0.001$), with the largest relative percent difference occurring in neutrophils (36%), and the lowest in eosinophils (14%).

Table 1. Comparison of mean number of leukocytes mm^3 by cell type in smokers and nonsmokers

	Cell type					
Group	White blood cells	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Smokers (803)*	8,177	4,897	2,439	478	206	61
Nonsmokers (2,145)*	6,319	3,606	2,029	387	181	50
Percent difference	+29%	+36%	+20%	+24%	+14%	+22%

*Number of participants in each group

A comparison of the percentage differential count in smokers and nonsmokers is shown in Table 2. Smokers had a significantly higher ($p < 0.001$) percentage of neutrophils and a significantly lower ($p < 0.001$) percentage of lymphocytes, a finding reported by others.[1, 4] In addition, the percentages of monocytes, eosinophils and basophils were each slightly, though significantly, ($p \leq 0.007$) lower in smokers compared to nonsmokers.

Table 2. Comparison of mean percentage of leukocytes mm^3 by cell type in smokers and nonsmokers

	Cell types					
Group	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	LUC**
Smokers (803)*	59.1	30.6	5.7	2.6	0.7	1.3
Nonsmokers (2,145)*	56.4	32.7	5.9	2.8	0.8	1.4

*Number of participants in each group

**Large unstained cells, type undetermined

Smoking also appeared to have an effect on platelets. Both male and female smokers had a slightly higher platelet count compared to nonsmokers (Table 3). Male smokers had a 3.2% higher platelet count than nonsmokers and female smokers a 5.1% higher count than nonsmokers.

Table 3. Comparison of mean platelet count in male and female smokers and nonsmokers

	Male		Female	
Group	Platelets/mm^3	Number of subjects	Platelets/mm^3	Number of subjects
Smokers	286,893	590	315,443	244
Nonsmokers	277,967	1,476	300,238	696
Significance (p)	$p = 0.019$		$p = 0.022$	

Table 4 gives the 95% "normal" range of cell types in both smokers and nonsmokers determined using a nonparametric method of percentile estimates. The values are derived from a cumulative frequency table at 2.5% and 97.5% giving the range of cell values which include 95% of the distribution. The leukocyte distribution in smokers is higher than the distribution for nonsmokers for every cell type in this table. In addition, the leukocyte distribution of smokers compared to nonsmokers has a greater variance, particularly for total WBC count, lymphocytes and neutrophils (Fig. 1-5). This may reflect a dose response relationship due to individual differences among smokers in the number of cigarettes smoked and the degree of inhalation.

Table 4. 95% range of values for leukocytes ($x10^3$ cells/mm^3) by cell type in smokers and nonsmoker*

Group	White blood cells	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Smokers	4.81-12.95	2.41-9.03	1.35-4.00	0.22-0.92	0.06-0.51	0.01-0.18
Nonsmoker	3.84-9.97	1.82-6.37	1.13-3.34	0.18-0.70	0.05-0.48	0.01-0.12

*Using the percentile estimates 2.5, 97.5

Discussion

We found a statistically significant difference in the absolute number of all five leukocyte cell types in smokers as compared to nonsmokers. Corre et al.[1] found similar differences in leukocyte count comparing smokers with non-smokers, and smokers who inhale with those who did not. In addition, we found that cigarette smoking had an effect on the differential white blood cell count, though not a major one. Smokers had only a slight increase in mean percentage of neutrophils, compared with nonsmokers, and a slight decrease in percentage of lymphocytes. This finding is in contrast to Parulkar et al.[14] who compared differential counts in 130 healthy Indian male smokers and nonsmokers finding a slight decrease in percentage of neutrophils in smokers and a statistically significant increase in percentage of lymphocytes ($p < 0.001$).

Different authors have proposed various explanations for how smoking may increase the leukocyte count. Friedman et al.[2] suggested that nicotine-induced catecholamine release might be a possible mechanism. Others have suggested that smoking may induce some kind of chronic inflammatory process like bronchitis which could possibly account for this increase in total leukocyte count.[14] A comparison of smokers who had a history of chronic bronchitis with smokers without such a history, showed that the increase in leukocyte count was mainly attributable to smoking and not to bronchitis.[1]

In the present study we found evidence that smoking has an effect on the platelet count. Both male and female smokers showed a significantly higher platelet count than their nonsmoking counterparts. A similar finding was reported by Erikssen et al.[13] for middle-aged men as long as 12 hours after smoking a cigarette.

With the use of the new Technicon H6000^{TM} blood cell analyzers which counts as many as 10,000 cells per sample, a more accurate picture of the effect of smoking on the leukocyte count is now available. Our study showed that cigarette smoking significantly increased all five cell types, including platelets. The mechanism by which this occurred is not known, but it is unlikely that a specific releasing or inducing factor is responsible for each of the different cell types. It did not appear the difference in total white blood cell counts between smokers and nonsmokers could be attributed to an allergy to cigarette smoke, since the percentage excess of eosinophils (a marker of many allergic reactions[15]) was less than that of the relatively nonspecific neutrophils. Furthermore, it did not appear to be simply an immune response to a foreign antigen in cigarette smoke, since the percentage excess of lymphocytes (a marker of reaction to a specific foreign antigen[15]) was less than that of neutrophils. Rather, it seems likely that a non-specific factor is present in cigarette smoke (or its metabolites) which may be responsible for this increase in the leukocyte and platelet counts found in smokers.

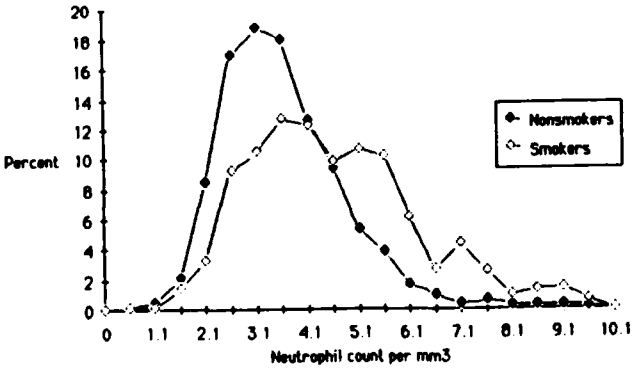

Figure 1. Frequency distribution of neutrophils in smokers and nonsmokers, Naval Weapons Center, China Lake, CA 1982

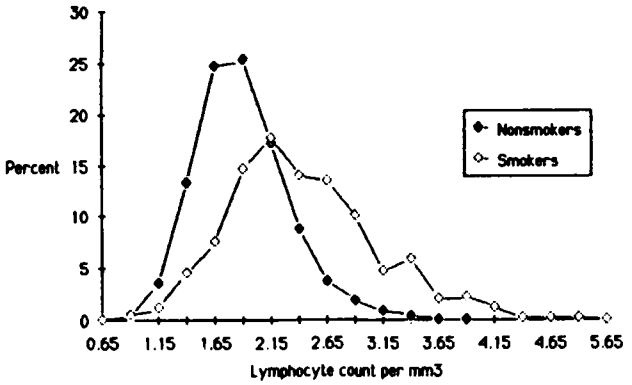

Figure 2. Frequency distribution of lymphocytes in smokers and nonsmokers, Naval Weapons Center, China Lake, CA 1982

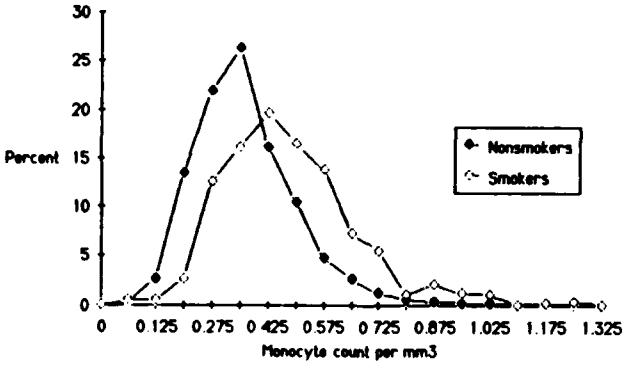

Figure 3 Frequency distribution of monocytes in smokers and nonsmokers, Naval Weapons Center, China Lake, CA 1982

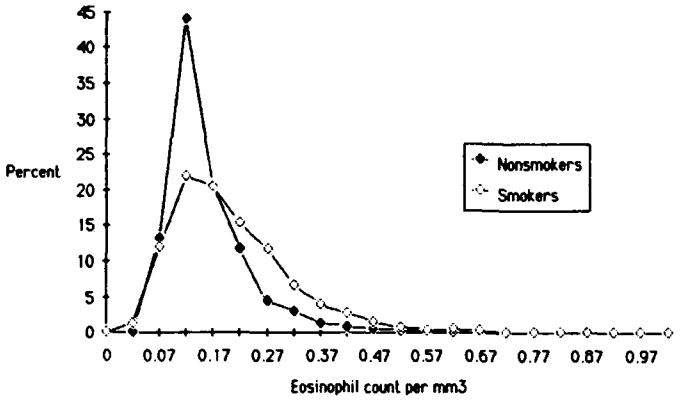

Figure 4. Frequency distribution of eosinophils in smokers and nonsmokers, Naval Weapons Center, China Lake, CA 1982

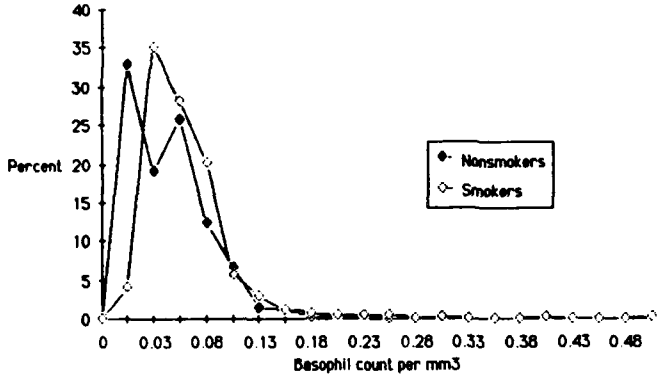

Figure 5. Frequency distribution of basophils in smokers and nonsmokers, Naval Weapons Center, China Lake, CA 1982

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A Technicon H6000™ automated blood cell analyzer was used to determine the effect of smoking on the differential white blood cell count and on platelet count. Approximately 3,000 apparently healthy individuals gave blood samples as part of an ongoing occupational health program. A significant increase in number of all leukocyte cell types was observed in smokers (8,177 cells per mm^3) as compared to nonsmokers (6319 cells per mm^3) ($p < 0.001$). The largest relative percent increase occurred in neutrophils (36%) and the lowest relative

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percent increase in eosinophils (14%). Smokers had a slight increase in mean percentage of neutrophils, compared with nonsmokers, and a slight decrease in mean percentage of lymphocytes. Smoking also appears to have affected the platelet count. Both male and female smokers show a slight increase in their platelet count in comparison to nonsmokers, 3.2% and 5.1% higher counts respectively. Possible explanations for the effect smoking has on the differential leukocyte counts are discussed.

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